16. Cellular Immune Response

Experiment MA-031

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ABSTRACT

Significant changes in the responsiveness of peripheral blood lymphocytes to phytohemagglutinin (PHA) occurred in the cellular immune response of three crewmembers during the 9-day flight of the Apollo-Soyuz Test Project. Parameters studied were white blood cell concentrations, lymphocyte numbers, B- and T-lymphocyte distributions in peripheral blood, and lymphocyte responsiveness to PHA, pokeweed mitogen, Concanavalin A, and influenza virus antigen.

INTRODUCTION

The health status of crewmembers before space flight has been a prime concern of NASA throughout the Apollo series of lunar landings and the Apollo-Soyuz Test Project (ASTP). The crews were selected long before the mission and were carefully monitored to determine their immunological responsiveness before flight. The humoral immune response was studied, and serum immunoglobulins (G, A, and M), C-3 complement component, and other serum proteins were quantitated (ref. 16-1). Significant elevations in IgA following space flight were reported. The cellular immune response was studied in 21 Apollo crewmembers for Apollo missions 7 to 13. Lymphocyte reactivity to phytohemagglutinin (PHA) (nonspecific mitogen) challenge only was reported by Fischer et al. (ref. 16-2). No significant change in lymphocytic responsiveness was reported. In comparison, in Skylab 3 and 4 studies, the functional capacity of the lymphocytes (again PHA responsiveness only) was depressed (ref. 16-3), and a suppression in the T-lymphocyte numbers was noted in this laboratory.

The cellular immune response of the three ASTP crewmembers was studied before and after the 9 days of flight. The findings are compared with lymphocytic changes that were noted during the Skylab mission. Briefly, as previously stated, the functional capacity of lymphocytes at splashdown of Skylab 3 and 4 was depressed along with a suppression in the T-lymphocyte numbers. In the ASTP study, which involved a shorter flight, functional suppression was again noted but no quantitative changes in lymphocytes occurred.

METHODS AND MATERIALS

Samples of heparinized peripheral venous blood (10 cm^3) were obtained from the three ASTP crewmembers and compared with similar samples from the three Skylab 4 crewmembers. In Skylab, no cultural studies were performed by this laboratory. All samples were processed within 1 to 24 hours after collection. Before separation, total leukocyte (white blood cell (WBC)) counts were performed using a hemacytometer and/or a Coulter counter, and differential counts were determined using slide preparations stained with Wright's stain.

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Lymphocyte Preparation

Lymphocytes were also separated by Ficoll-Hypaque gradient centrifugation according to Böyum's method (ref. 16-4) or by using a Technicon lymphocyte separator. Cells in the resulting suspension of mononuclear cells were washed three times in minimum essential medium (MEM); they were then adjusted to a final concentration of 1×10^6 cells/ml.

Lymphocyte Classification

The B-lymphocyte distributions were determined by enumerating the percent of 200 mononuclear cells with surface immunoglobulins detected by the immunofluorescent antibody technique described in reference 16-5.

The E-rosette-forming lymphocytes (T-cells) were determined by the method of Jondahl et al. (ref. 16-6). A concentration of 1×10^6 lymphocytes was mixed in 0.25 ml of MEM and added to 0.25 ml of a 0.5-percent sheep red blood cell (SRBC) suspension. After mixing, the tubes were incubated at 310 K (37° C) for 5 minutes, centrifuged at 500g for 3 minutes, and incubated in ice water for 2 hours. Approximately half the supernatant was removed, and the top layer of cells was gently resuspended; 200 lymphocytes were counted with 3 or more adhering SRBC's used as criteria for E-rosetted lymphocyte.

Lymphocyte Responsiveness in Microculture to PHA, PWM, and Con A

Purified lymphocytes (1×10^5 cells) in MEM containing 40-percent fetal calf serum (FCS) were placed in culture plates, and the antigens were diluted in MEM (containing Pen-Strep and L-glutamine) in the following concentrations: 0.01 ml PHA/ml MEM; 0.01 and 0.05 ml pokeweed mitogen (PWM)/ml MEM; and 25 and 50 μg Concanavalin A (Con A)/ml MEM. The diluted antigens in 0.1-ml aliquots (in duplicate) were placed in the appropriate well. The cultures were incubated in a humidified carbon dioxide atmosphere at 310 K (37° C) for 3 days for PHA and 5 days for Con A and PWM. On the day of harvest, samples were treated as described in the following section on the influenza virus antigen cultures.

Lymphocyte Responsiveness to Influenza Virus Antigen by Thymidine Incorporation

An inactivated monovalent type A influenza virus vaccine containing 1600 chick cell agglutinating units/ml of a Hong Kong strain (H_3N_2) was dialyzed against phosphate-buffered saline (PBS) and stored at 203 K (−70° C) until used for tests. Separated lymphocytes were added in 0.1-ml aliquots (in duplicate) for each dilution of influenza antigen, and the cultures were incubated in a humidified carbon dioxide incubator at 310 K (37° C) for periods of either 2 or 3 days. On the day of harvest, the cells were pulsed for 2 hours with 37 billion disintegrations/sec (1 μCi) methyl ^{3}H-thymidine and then harvested with an automated harvester onto glass-fiber filter strips; they were then counted in a liquid scintillation counter. Data were expressed as counts per minute (CPM)/1×10^6 lymphocytes, and the stimulation index (SI) was calculated by dividing the mean result for stimulated cultures by that for the unstimulated cultures. Allantoic fluid was used separately as a control on the reactivity of each individual crewmember to chick embryo proteins.

RESULTS

White Blood Cell Quantitation

For the ASTP and the Skylab 4 crewmembers, table 16-I shows the mean and 1 standard deviation of the WBC count and the lymphocyte count for respective days preflight and postflight. Normal laboratory values are given for comparison. In the ASTP crew, significant increases in WBC numbers were noted on R+0 (day of splashdown) and R+1, which represented polymorphonuclear leukocyte increases. Individual variation in WBC

TABLE 16-I.—Preflight and Postflight Leukocytic Response of ASTP and Skylab 4 Crewmembers

Sample day (a)	*WBC/mm³, mean ± 1 SD[b]*	*Lymphocyte count/mm³, mean ± 1 SD*
	ASTP	
F−30	6 640 ± 1830	2820 ± 480
F−15	5 830 ± 2020	2420 ± 440
F−5	6 530 ± 560	3040 ± 550
R+0	11 110 ± 4210	1950 ± 490
R+1	13 530 ± 610	3020 ± 810
R+8	9 560 ± 2580	3500 ± 900
R+13	6 600 ± 1510	2270 ± 400
Normal laboratory value	7 110 ± 2400	3000 ± 1200
	Skylab 4	
F−30	6013 ± 254	1940 ± 670
F−15	5243 ± 699	1820 ± 213
F−7	5903 ± 1517	2023 ± 699
F−1	5206 ± 645	1987 ± 206
R+0	8980 ± 5560	1177 ± 381
R+1	6230 ± 1265	2757 ± 349
R+3	7370 ± 1528	2280 ± 479
R+7	5977 ± 1498	2320 ± 585
R+14	4840 ± 881	1827 ± 118

[a]F−30 is 30 days before lift-off, R+0 is recovery (splashdown) day, R+1 is recovery plus 1 day, etc.

[b]SD = standard deviation.

values (a range of 3300 to 14 410) was noted among the three Skylab 4 crewmembers at R+0, which is reflected in the large standard deviation in table 16-I. However, all values were within clinically normal ranges. Less variation was noted for total lymphocyte values, as reflected by the mean and standard deviation shown in table 16-I. All values at R+0 were decreased below their previous baseline values.

The results of the T-lymphocytes, B-lymphocytes, and nonreactive lymphocytes of ASTP crewmembers are given in table 16-II. Fluctuations are indicated, but values remain within the 1 standard deviation for normal laboratory values. In contrast, highly significant changes were noted in B and T cell distributions of the Skylab 4 crewmen (fig. 16-1).

Lymphocyte Responsiveness in Culture to PHA, PWM, and Con A

Cultural studies were performed on samples from the ASTP mission only. The culture findings for the nonspecific mitogens are shown in table 16-III. The stimulation indexes for PHA dropped at R+0 and at R+1 for all three crewmembers. By R+8, the three crewmembers were back within normal ranges. Interestingly, a reverse situation is indicated for the Apollo commander (ACDR) at R+0 with Con A stimulation enhanced. Other crewmembers began and remained at low-level ranges for Con A throughout the preflight and postflight periods. Considerable individual variation was noted in the PWM data and no consistent trend was obtained.

TABLE 16-II.—Results of the T-Lymphocytes, B-Lymphocytes, and Nonreactive Lymphocytes

Sample day	*T-lymphocytes, 1×10^5/ml (mean ± 1 SD)*	*B-lymphocytes, 1×10^5/ml (mean ± 1 SD)*	*Nonreactive lymphocytes, 1×10^5/ml (mean ± 1 SD)*
F−30	16.5 ± 6.1	7.1 ± 0.4	4.7 ± 2.1
F−15	12.9 ± 4.9	4.7 ± 0.2	6.7 ± 0.5
F−5	17.9 ± 2.8	5.4 ± 0.9	7.1 ± 3.7
R+0	13.0 ± 5.6	5.3 ± 1.2	1.2 ± 1.2
R+1	19.1 ± 3.8	7.0 ± 3.5	4.2 ± 1.8
R+8	25.3 ± 4.4	5.8 ± 1.9	3.9 ± 3.6
R+13	14.4 ± 3.2	5.6 ± 1.2	2.6 ± 1.0
Normal laboratory value	15.9 ± 6.3	5.1 ± 3.0	8.5 ± 5.6

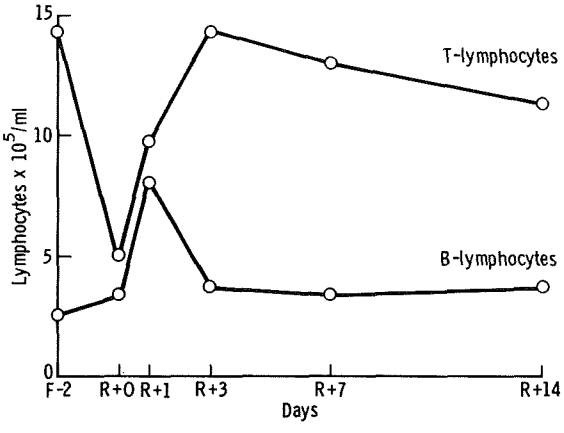

FIGURE 16-1.—Mean Skylab 4 B- and T-lymphocyte responses preflight and postflight.

Influenza Virus Antigen Stimulation

One of the three ASTP crewmembers responded to influenza virus Hong Kong strain antigen in cultures. All three astronauts responded to influenza virus England strain preflight. Because these antigens are quite variable in response patterns even in known and diagnosed influenza illnesses, changes or losses in reactivity are not completely understood. Total lack of reactivity may reflect influenza susceptibility.

DISCUSSION

Significant changes were found for all three ASTP crewmembers in the responsiveness of the lymphocytes to PHA. No significant quantitative changes were noted among the lymphocytic population. Variations were noted in the stimulation indexes of the subjects for Con A and PWM; however, because of the rapid exchange of cells between different compartments in the body, variation in the indexes may be expected. Again, an overall suppression at R+0 was found.

The ASTP crewmembers experienced contact with toxic gases upon splashdown. The exposure was severe enough to create concern for lung inflammation; this concern resulted in corticosteroid treatment on R+1. Samples were collected on R+0 before the administration of the steroid. Consequently, it is likely that the increased immunosuppressions noted by cultural responses on R+1 for all mitogens and on R+8 for Con A were attributable to the steroid therapy. Most cortisone preparations are known to affect the lymphocytic reaction. The depressions observed at R+0 were due to either space flight or possibly the toxic gas exposure a few hours before the analysis. No data are available on the effects of toxic gases on lymphocytic responses. Similar depressions in lymphocytic responsiveness were observed in Skylab 3 and 4 data; both of these missions were of much longer duration than ASTP

TABLE 16-III.—Lymphocytic Response to PHA, PWM, and Con A

Sample day	*Subject (a)*	*Stimulation indexes*		
		PHA	*Con A*	*PWM*
F−30	ACDR	34	3	5
	CMP	34	26	50
	DMP	41	16	114
	Mean of three	36 ± 4	15 ± 12	56 ± 55
F−15	ACDR	65	25	43
	CMP	47	11	14
	DMP	95	12	41
	Mean of three	69 ± 24	16 ± 8	33 ± 16
F−5	ACDR	66	12	27
	CMP	46	32	29
	DMP	74	6	34
	Mean of three	63 ± 15	17 ± 14	30 ± 4
R+0	ACDR	38	151	100
	CMP	11	11	24
	DMP	12	12	11
	Mean of three	20 ± 15	58 ± 80	45 ± 48
R+1	ACDR	9	20	40
	CMP	21	49	11
	DMP	5	6	4
	Mean of three	12 ± 8	25 ± 22	25 ± 15
R+8	ACDR	72	12	11
	CMP	45	18	11
	DMP	18	8	9
	Mean of three	45 ± 27	13 ± 5	10 ± 1
Normal laboratory value	Mean	69	67	50
	Range	10 to 282	10 to 277	10 to 170

[a]ACDR, CMP, and DMP indicate Apollo commander, command module pilot, and docking module pilot, respectively.

(ref. 16-3). However, in more comparable missions of similar lengths (the Apollo series) no changes in lymphocytic responsiveness were reported (ref. 16-2).

The ASTP crewmembers showed no change in the quantity of B- or T-lymphocytes present in the peripheral blood. In contrast, the Skylab 4 crewmembers showed significant change. The principal difference between the two missions appeared to be due to the length of time in space. Various disease states have been shown to exhibit proportional as well as quantitative changes in these cells. Although the crewmembers appeared to have no overt disease processes upon return, they did appear to experience a similar stress phenomenon on the body to create the depression observed in lymphocytes. Because no in-flight blood samples were analyzed for lymphocytic responsiveness, it is not known if the suppression begins immediately after a 9-day stay in space or is a result of splashdown stresses. For Skylab 3, Leach et al. (ref. 16-7) reported increased in-flight urinary cortisol levels compared with baseline values. The greatest increase was immediately upon launch; however, values remained increased throughout the flight. Because cortisol, in high doses, is known to control the immune response, the depressions in lymphocytic responsiveness in this instance may be related to *in vivo* cortisol control.

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